

## *Coxiella burnetii* in samples from cattle herds and sheep flocks in the Kars region of Turkey

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**ABSTRACT:** This study was aimed at determining the presence of *C. burnetii* in cattle and sheep herds in the Kars region of Turkey using serological and molecular methods. As a serological technique, *C. burnetii* IgG in blood sera and milk samples were investigated with ELISA. The results of these examinations revealed that 108 (43.2%) out of 250 sheep blood serum samples and 52 (14.85%) out of 350 cattle blood serum samples were seropositive for *C. burnetii* antibodies by ELISA. Out of 350 cattle and 250 sheep milk samples examined with ELISA, 36 (10.28%) and 42 (16.8%) were found to be seropositive, respectively. For molecular analysis, a Trans-PCR amplifying the *IS1111A* transposase gene of *C. burnetii* was conducted. Five (1.42%) out of 350 cattle milk samples, and one (0.4%) from 250 sheep milk samples were found to be positive for *C. burnetii* DNA. The results obtained in this study have demonstrated the presence of Q fever in cattle and sheep in the Kars region, and the dissemination of the infectious agent within milk. This situation poses a potential risk for animal and human health. Ultimately, this study points the way to future investigations into the presence of *C. burnetii*, which causes Q fever in cattle and sheep, and will contribute to the protection and control of the disease.

**Keywords:** *Coxiella burnetii*; Q fever; cattle; sheep; sera; ELISA; PCR

The causative agent of Q fever is the intracellular obligate Gram-negative bacterium *Coxiella burnetii* (Drancourt and Raoult 2005; Kilic and Celebi 2008; Angelakis and Raoult 2010). Q fever is one of the most common zoonotic diseases in the world (Maurin and Raoult 1999). The causative agent has a wide host spectrum, including ruminants such as cattle, sheep, and goats, as well as cats and dogs, and occasionally birds, ticks and humans (Ozgur et al. 1997; Hartzell et al 2008). Although many species are known to be susceptible to infection, the majority of infected animals are asymptomatic. Infected animals may rarely present with fever, conjunctivitis, arthritis, mastitis, abortion and genital disorders (Aitken 1989; Berri et al. 2002).

*Coxiella burnetii* does not grow in standard media. Its isolation is time-consuming, burdensome and dangerous. As it is highly infectious and requires biosafety level three conditions for its handling, *C. burnetii* is only infrequently subjected to routine diagnosis in veterinary medicine (Fournier

et al. 1998). For the laboratory diagnosis of *C. burnetii*, either direct identification methods, including the isolation of the causative agent from clinical samples, molecular techniques such as polymerase chain reaction (PCR), and immunohistochemical techniques, or indirect serological methods like enzyme-linked immunosorbant assay (ELISA), based on the demonstration of the presence of antibodies against the agent, are used (Fournier et al. 1998; Kovacova and Kazar 2002; Thompson et al. 2005; Kilic and Celebi 2008).

Given the high infectivity of *C. burnetii* and due to the fact that isolation techniques are costly, time-consuming and lacking in sensitivity, serological tests are preferentially used for the confirmation of clinical diagnosis (Berri et al. 2000; Raoult et al. 2001; Kilic and Celebi 2008). It is considered that the serological and molecular diagnosis of Q fever, known to cause major economic losses in the livestock sector, which is one of the main sources of income in the study area, would contribute to

determining the prevalence of the disease. As very little research has been conducted on Q fever in the study area, and since the public lacks information on the risks associated with this disease, the significance of Q fever remains unclear. This study was aimed at determining the presence of Q fever in cattle herds and sheep flocks, and at demonstrating the significance of the disease.

## MATERIAL AND METHODS

**Study area.** This study was carried out in the province of Kars located in the north-eastern area of Anatolia (Figure 1).

**Material.** In total, 600 milk and 600 blood samples taken from 350 cattle and 250 sheep, raised in villages in central Kars province, constituted the material of the study. Samples were collected at the same time from each animal. Prior to the collection of the milk samples, the teats were cleansed with soapy water and alcohol. The fore-milk was discarded and milk samples of approximately 40-ml volume were collected into 50-ml sterile screw-capped Falcon tubes. From the milked cattle, blood samples of 7–8 ml volume were also taken into 10-ml sterile blood tubes (A.D.R vacuum tube, Lot: 20101208).

**ELISA.** The sera, obtained from the blood and milk samples, were serologically tested for the presence of anti-*C. burnetii* antibodies. For this purpose, a Q-fever (*C. burnetii*) antibody test kit

(Chekit Q Fever, Idexx Lab., Zul.-Nr.: BGAF-B-101) was used, and the test was performed in accordance with the instructions of the manufacturer. The results were read using an ELISA reader at a wavelength of 450 nm. For the calculation of the ELISA results, the following formulation was used: Value (%) =  $[\text{OD sample} - \text{OD neg} / \text{OD pos} - \text{OD neg}] \times 100\%$ . Results, which were < 30%, were considered to be negative, whilst those, which were  $\geq 30\%$  to < 40%, were considered suspicious, and those that were  $\geq 40\%$  were assessed to be positive.

## PCR

**DNA extraction.** In total, 600 milk samples, 350 of which were collected from cattle and 250 of which were collected from sheep, were tested using molecular methods. A QIAamp DNA tissue and blood kit (Qiagen S.A, France, Lot. No: 142328642) was used for DNA extraction. DNA extraction from the milk samples was performed in accordance with the manufacturer's instructions.

**DNA amplification.** To the reaction mixture, prepared at a volume of 22  $\mu\text{l}$  in 0.2-ml test tubes for each sample, was added 3  $\mu\text{l}$  of the DNA extract, and amplification was performed as described by Berri et al. (2000) using the standardised touch-down polymerase chain reaction (PCR) protocol. In this study, the *C. burnetii* Nine-Mile I strain was used as the positive control and ddH<sub>2</sub>O was used as

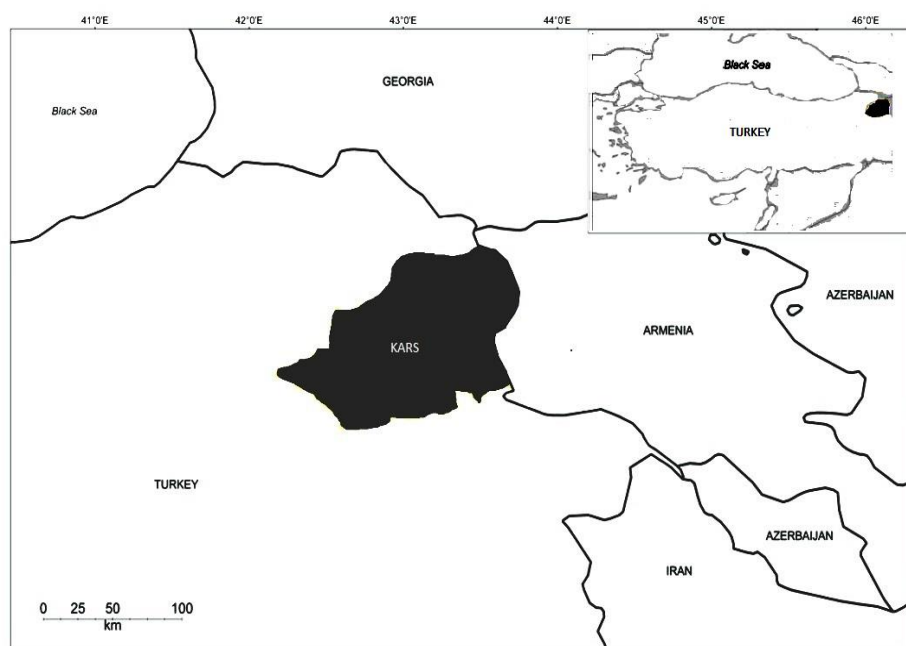


Figure 1. Location of Kars Province in Turkey

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Table 1. Positive results in blood and milk samples tested using ELISA for *C. burnetii* antibodies

Species	Number of tested samples	Number of positive serums	Number of positive milk
Cattle	350	52	36
Sheep	250	108	42
Total	600	160	78

the negative control. For the *IS1111A* transposase gene, which is 687 bp in length and is specific to the DNA of *C. burnetii*, Trans-I (5'-TAT GTA TCC ACC GTA GCC AGT C-3') and Trans-II (5'-CCC AAC AAC ACC TCC TTA TTC-3') primers were used. Following amplification, electrophoresis was performed in 1.5% agarose gels, and the results were imaged.

## RESULTS

### ELISA findings

In this study the presence of anti-*C. burnetii* immunoglobulin G (IgG) was investigated. Out of the 350 bovine blood serum samples tested, 52 (14.85%), and out of the 250 ovine blood serum samples test-

ed, 108 (43.2%), were found to be positive. Out of the 350 bovine milk samples tested, 36 (10.28%), and out of the 250 ovine milk samples, 42 (16.8%), were determined to be positive (Table 1).

### PCR findings

The *IS1111A* transposase gene, 687 bp in length, was detected using Trans-PCR in five (1.42%) of the 350 bovine milk samples collected from cattle herds raised in the villages of the central region of Kars province. The Trans-PCR results obtained in the present study demonstrated that, out of the 250 ovine milk samples, only one (0.4%) was positive (Figure 2).

## DISCUSSION

Owing to its localisation to both the uterus and the mammary glands, in animals, *Coxiella burnetii* leads to reproductive symptoms, abortion or infertility (Arserim et al. 2011). Diagnosis is of key importance to the control of Q fever (Kovacova and Kazar 2000; Raoult et al. 2001). Cattle, sheep and goats act as the primary reservoirs in the transmission of Q fever to humans (Maurin and Raoult 1999). The shedding of the microorganism into the environment, and in the milk, urine, faeces, placenta and foetal membranes of infected animals, plays a role in the transmission of infection to humans (To et al. 1995). Epidemiological data suggests that chronic infections are more common in dairy cattle than in sheep (Maurin and Raoult 1999). Reports indicate that, compared to sheep, in cattle, specific antibodies can be detected for a longer time period in serum samples, and *C. burnetii* is shed in milk for a longer period of time (To et al. 1995). In a study in which milk samples of 44 dairy cattle herds raised in southern Iran were taken from milk tanks and tested using ELISA, Khalili and Sakhaee (2009) detected a positivity rate of 45.4%. On the other hand, Agger et al. (2010) reported a positivity rate of 59% in randomly selected cattle herds in Denmark. Based on the ELISA analysis of milk and blood serum samples of 448 cattle from six different herds raised in France, Guatteo et al. (2007) detected the presence of anti-*C. burnetii* antibodies in 264 of the blood serum samples and in 257 of the milk samples.

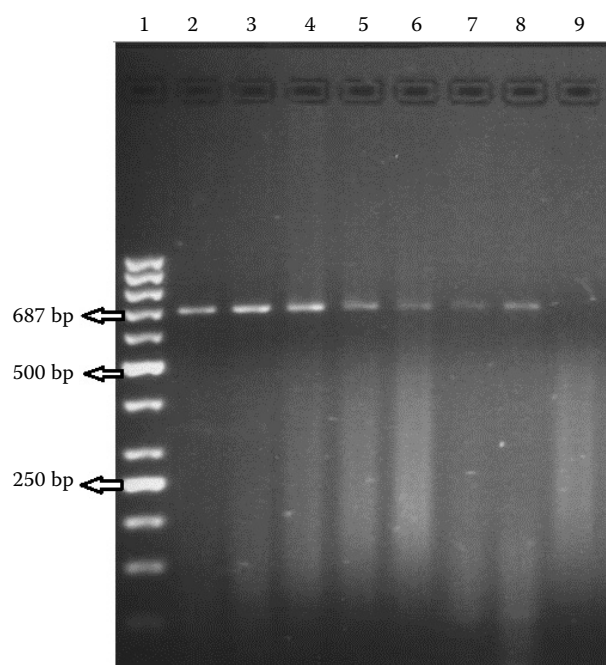


Figure 2. Gel-electrophoresis image of amplification of the *IS1111A* transposase gene using Trans-PCR

1 = 50 bp DNA Ladder, 2 = positive control, 3–7 = cattle samples, 8 = sheep sample, 9 = negative control

Seyitoglu et al. (2006) reported a seropositivity rate of 9.5% in cattle raised in Erzurum province and its vicinity. In their study, the researchers detected positivity in 12 (22.6%) out of 53 cattle with a history of abortion, and in 10 (5.6%) out of 177 cattle with no history of abortion. Ozturk et al. (2012) reported a seropositivity rate of 19 (10.2%) out of 186 dairy cattle with a history of abortion in Burdur province. Based on the ELISA analysis of blood serum samples, Karaca et al. (2009) detected seropositivity in 98 (21.07%) out of 465 sheep. Furthermore, Kalender (2001) reported a seropositivity rate of 38.59% in sheep with a history of abortion, and a rate of 11.01% in sheep with no history of abortion in Elazig and its neighbouring provinces.

Leloglul (1977) investigated the presence of Q fever in humans, cattle and sheep in Erzurum, Kars and Agri provinces using the microagglutination (MA) test, the capillary tube agglutination (CA) test and the complement fixation test (CFT). In total, this researcher tested 456 ovine and 262 bovine serum samples taken from these three provinces. He reported a Q fever detection rate of 22.1% in ovine serum samples, 15.6% in bovine serum samples and 11.2% of 178 human serum samples obtained from the Biochemistry laboratory of Erzurum Numune Hospital. The assessment of the results with respect to the provinces included in the study demonstrated that positivity was detected in 50 (23.4%) out of the 209 ovine serum samples taken from Erzurum province, in 30 (20.7%) out of the 145 ovine serum samples taken from Kars province, and in 21 (20.6%) out of the 102 ovine serum samples taken from Agri province. On the other hand, 13 (12%) out of the 108 bovine serum samples taken from Erzurum province, 18 (17.1%) out of the 105 bovine serum samples taken from Kars province and 10 (20.4%) out of the 49 bovine serum samples taken from Agri province were determined to be positive for Q fever.

In the present study, which was conducted in Kars province, a seroprevalence of 14.85% was detected on the basis of the analysis of 350 bovine serum samples using a commercial ELISA test kit. The seroprevalence detected for cattle in the present study was found to be in accordance with the results of previous studies conducted in cattle throughout the country and in the study area. No significant differences were detected between the results of the present study and previous research. Furthermore, of the 250 ovine serum samples tested

in the present study, 43.2% were found to be positive. Compared to previous research, the seropositivity rate detected in the present study for sheep was found to be higher. This high seropositivity can be attributed to geographical biodiversity, breeding and management conditions, and inadequate control of ticks and other arthropods.

Although serological methods are considered to be useful in the diagnosis of Q fever in animals, these methods present with some disadvantages in the detection of animals infected with *C. burnetii*, which shed the microorganism in their vaginal mucus, faeces and milk. Although infected animals, that shed the microorganism in their faeces, milk and vaginal mucus, are mostly detected as seropositive, not all seropositive animals necessarily shed the agent, and conversely, the agent may be shed by some animals that are, in fact, seronegative (Fournier et al. 1998; Berri et al. 2001; Arricau-Bouvery and Rodolakis 2005). Several PCR techniques have been developed for the identification of *C. burnetii* DNA in clinical samples and cell cultures. Trans-PCR, which involves the use of primers based on the transposon element of *C. burnetii*, is a highly specific and sensitive diagnostic method (Ozgur et al. 1997; Fournier et al. 1998; Brennan and Samuel 2003). These primers target the *IS1111A* transposase gene, which is present in 20 copies in the genome of the Nine Mile RSA 493 strain for *C. burnetii*. This particular PCR technique is highly sensitive, as the *IS1111* element has a large number of copies, and because the similarity of the sequence of this element with the sequences of other microorganisms is rather limited (Hoover et al. 1992; Masala et al. 2004; Klee et al. 2006; Kirkan et al. 2008).

In their study, in which they tested 138 blood samples taken from various cattle farms using PCR, Kirkan et al. (2008) found six samples (4.3%) to be positive. Berri et al. (2009) analysed 253 clinical samples taken from ruminant herds using the m-PCR technique and specific primers, and detected the presence of *C. burnetii* in 49 of the samples (33 vaginal swabs, 11 milk samples, four faeces samples, and one placenta sample). In a study conducted in Northern Cyprus, Cantas et al. (2011) detected *C. burnetii* in 22 out of 59 foetal abomasal content samples using the Trans-PCR technique. Kargar et al. (2013) analysed 100 bovine milk samples taken from milk tanks using PCR and reported 11 of them to be positive. With the aim of describing



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the dissemination and serological development of *C. burnetii* in animals with a history of abortion associated with this microorganism, Guatteo et al. (2012) monitored 24 cows for a one month period, and analysed milk and vaginal mucus samples, collected weekly after the occurrence of abortion, using PCR and ELISA techniques. These researchers ascertained that, while the dissemination of the microorganism continued for only a very short time period in vaginal mucus following abortion, the microorganism was disseminated in milk for a longer period of time.

In the present study, out of the 350 bovine milk samples and 250 ovine milk samples collected from villages from the central region of Kars province, 1.42% and 0.4% were found to be positive using the trans-PCR technique, respectively. The prevalence rates detected for the bovine and ovine milk samples with the use of the trans-PCR technique in the present study are lower than the positivity rates determined in previous reports. This can be attributed to differences in the sampling period and the analysis methods used. Furthermore, we found the prevalence in cattle to be higher than that determined in sheep.

Pursuant to the permission granted with the decision of the Animal Experiments Local Ethics Board of Kafkas University, taken at its 13<sup>th</sup> meeting held on 15.07.2010, blood and milk samples were taken from cattle and sheep.

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